SYNOPSIS OF RESEARCH PROJECT

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8.	**Title of Research Project**	**:**	**NANOPARTICLE-MEDIATED INDUCTION OF DEFENSE RESPONSES IN GROUNDNUT (*Arachis hypogaea*) AGAINST *Aspergillus niger* van Tieghem CAUSING COLLAR ROT**

9. Introduction

India occupies an important position in the global oilseed economy and oilseed crops form a vital component of its agricultural sector. The country cultivates nine major oilseed crops namely groundnut, rapeseed-mustard, soybean, sunflower, safflower, sesame, niger, castor and linseed contributing significantly to edible oil production and nutritional security. In recent years 2025-26 oilseed production in India has improved due to expansion in cultivated area, adoption of improved cultivars and better crop management practices, with Gujarat contributing substantially to national output.

Groundnut or peanut (*Arachis hypogaea* L.) is one of the most important leguminous oilseed crops grown in tropical and subtropical regions of the world. The crop originated in Brazil, South America and is currently cultivated in more than 80 countries between 40°N and 40°S latitudes (Kumbhar and Deshmukh, 2025). In India, groundnut occupies about 47.07 lakh hectares with a production of 101.80 lakh tonnes during 2023-24 (Anonymous, 2024). Gujarat is the leading groundnut-producing state in the country, covering nearly 2.0-2.2 million hectares with production exceeding 4.0 million tonnes during 2024-25 (Anonymous, 2025). Major producing districts include

Junagadh, Rajkot, Amreli, Jamnagar, Bhavnagar and Porbandar, with the Saurashtra region accounting for a major share of the acreage.

Groundnut belongs to the family Fabaceae and possesses a unique geocarpic habit where pods develop underground (Pattee and Stalker, 1995). The crop is nutritionally rich containing about 45-50 per cent oil and 25-30 per cent protein along with vitamins and minerals and is therefore often referred to as the "King of Oilseed Crops" (Reddy, 1976). Being a legume, it forms a symbiotic association with *Rhizobium* species and fixes about 80-160 kg nitrogen per hectare per season, thereby improving soil fertility (Alam *et al*., 1988).

Despite its economic importance, groundnut productivity is affected by several biotic stresses particularly fungal diseases such as early leaf spot (*Cercospora arachidicola*), late leaf spot (*Phaeoisariopsis personata*), rust (*Puccinia arachidis*), collar rot (*Aspergillus niger*), stem rot (*Sclerotium rolfsii*) and root rot (*Macrophomina phaseolina*) (Mc Donald *et al*., 1985). Among these, collar rot caused by *Aspergillus niger* van Tieghem is one of the most destructive seed and soil-borne disease especially during the *Kharif* season. The disease was first reported by Jochem (1926) and later recorded in India by Jain and Nema (1952). In Gujarat, particularly in the Saurashtra region, collar rot has become a serious problem under high temperature (around 30°C) and low soil moisture conditions (Patel *et al*., 2015). The site of infection by this pathogen on groundnut is radicle, cotyledons and also affect collar region near the soil line causing rotting, wilting and death of the plant. Yield losses may range from 10-50 per cent depending on environmental conditions and cultivar susceptibility (Pande and Rao, 2000; Shaheen, 2018). The pathogen survives in soil and crop debris as mycelium, conidia and infected seed acts as a primary source of inoculum (Nema *et al*., 1955).

Management of collar rot mainly relies on chemical seed treatment with fungicides such as tebuconazole, carboxin, thiram and mancozeb (Rakholia *et al*., 2012). However, excessive use of agrochemicals may lead to environmental contamination and resistance development, highlighting the need for eco-friendly disease management approaches (Rakholia and Jadeja, 2010). In this context, nanotechnology has emerged as a promising tool in modern agriculture (Hazarika *et al*., 2022). Nanotechnology involves the manipulation of materials at the nanoscale (1-100 nm) and offers unique physicochemical properties such as high surface area and enhanced reactivity (Singh, 2020). Nano-based products including nanofertilizers,

nanopesticides and nanosensors have shown potential in improving crop productivity and plant disease management (Prasad *et al*., 2017; Mohana *et al*., 2020).

Green synthesis of nanoparticles using plant extracts has gained considerable attention as an eco-friendly alternative to conventional synthesis methods. Plant extracts containing biomolecules such as phenols, flavonoids, terpenoids and proteins act as reducing and stabilizing agents during nanoparticle formation (Mittal *et al*., 2013; Chand *et al*., 2019). Among various metal nanoparticles, silver and iron nanoparticles have shown strong antimicrobial activity against several plant pathogenic fungi. Silver nanoparticles inhibit spore germination and mycelial growth, while iron nanoparticles are considered relatively eco-friendly and may also enhance plant growth and nutrient availability. These nanoparticles have shown promising results in suppressing *Aspergillus niger* even at lower concentrations compared to conventional fungicides (Scroccarello *et al*., 2021; Shi *et al*., 2022).

10. Practical Utility of the Research Project

Collar rot of groundnut caused by *Aspergillus niger* is a serious seed and soil-borne disease in major groundnut-growing regions of Gujarat, particularly under high temperature and low soil moisture at seedling stage. The disease causes severe seedling mortality and yield losses ranging from 10-50 per cent (Pande and Rao, 2000; Shaheen, 2018). Collar rot is more serious problem in sandy soil (Gibson, 1953; Chohan, 1965). At present, management mainly depends on chemical fungicides which may lead to environmental contamination, pathogen resistance and increased cost of cultivation. Therefore, there is a need to develop eco-friendly and effective alternatives for disease management. In this context, green synthesized silver and iron nanoparticles offer promising potential due to their strong antifungal activity, higher efficacy at lower concentrations and environmentally safe nature.

Hence, the present study aims to explore the synthesis, characterization and antifungal potential of plant extract mediated silver and iron nanoparticles for the management of collar rot of groundnut caused by *Aspergillus niger*.

11. Objectives

The present investigation on "Nanoparticle-mediated induction of defense responses in groundnut (*Arachis hypogaea*) against *Aspergillus niger* van Tieghem causing collar rot" was undertaken with the following objective

1. To isolate, purify, identify and confirm the pathogenicity of fungus causing collar rot of groundnut

2. To synthesize and characterize nanoparticles for their physiochemical properties
3. To investigate the biochemical changes induced in groundnut upon nanoparticle treatment
4. To investigate role of epidemiological factors on disease development
5. To evaluate the efficacy of different nanoparticles (AgNPs, FeNPs) against *Aspergillus niger* under *in vitro* condition
6. To evaluate the efficacy of selected fungicides and nanoparticles in reducing disease incidence in groundnut under field conditions

12. Review of literature

In the present investigation, emphasis is given to the green synthesis and characterization of silver and iron nanoparticles and their effect on collar rot of groundnut caused by *Aspergillus niger* van Tieghem. The available literature related to nanoparticle synthesis, characterization and their antifungal activity, particularly for the management of collar rot in groundnut (*Arachis hypogaea*), has been reviewed and presented under the following sub-headings.

12.1 Isolate, purify, identify and confirm the pathogenicity of fungus causing collar rot of groundnut.

Early investigations on species of *Aspergillus niger* revealed that several environmental and biological factors influence the germination of fungal conidia. Jain and Nema (1952) reported that parameters such as inoculum concentration, age of spores, and temperature play an important role in determining the germination behavior of *Aspergillus* conidia.

Okuda *et al.* (2000) isolated fungal colonies from groundnut plants that showed rapid growth with colony colours ranging from white, yellow, yellow-brown, brown to black or green shades**.** These colonies, characterized by dense growth of erect conidiophores, were broadly categorized as *Aspergillus* species.

Youssef *et al.* (2008) obtained a pure culture of *Aspergillus niger* through tissue isolation from collar rot-infected groundnut plants.

Bobbarala *et al.* (2009) reported that *Aspergillus niger* causes several plant diseases, including root and stalk rot of *Sansevieria*, boll rot of cotton, and spoilage of cashew kernels, dates, figs, vanilla pods and dried prunes.

Gajera *et al*. (2011) reported that collar rot of groundnut occurs in two stages: pre-emergence and post-emergence. In the pre-emergence stage, infected seeds rot in the soil or show dark spore masses during germination, leading to death of the emerging

hypocotyl. In the post-emergence stage, small light-brown lesions appear on cotyledons and later spread to the hypocotyl or stem region, resulting in tissue rotting and eventual collapse of the seedlings.

Mohapatra and Beher (2012) isolated, purified and proved pathogenicity of five isolates of *Aspergillus niger* in groundnut collected from different agro-ecological situations. They recorded lower seed germination (60.33 to 75.00) in artificially inoculated seeds and 16.66 to 33.00 per cent seed rot

According to Rakholia *et al.* (2012) collar rot of groundnut is more severe in sandy loam and medium black soils. Their findings also indicated that most cultivars of *Arachis hypogaea* are susceptible to this disease, which appears during both pre-emergence and post-emergence stages. Infected seeds often turn dark and fail to germinate, while seedlings that emerge show rotting of the collar region followed by wilting and eventual death.

Radhaiah and Nagalakshmi (2013) isolated the pathogen from groundnut plants affected by collar rot showing wilting symptoms and rotting of tissues near the soil surface. The infected tissues appeared dark, shrunken, and later became covered with black masses of fungal spores. Microscopic examination revealed upright conidiophores ending in globose vesicles, with one-celled globose conidia ranging from light to dark brown. The mycelium was hyaline, septate, and branched, confirming the fungus as *A. niger*.

Kumari and Singh (2016) studied the pathogenic and morphological variability of *Aspergillus niger* isolates on groundnut. Four isolates (AN-01, AN-02, AN-03 and AN-04) were evaluated and showed significant differences in pre- and post-emergence mortality.

Furthermore, Shaheen (2018) reported that collar rot is particularly severe during the early stages of crop growth and can result in significant seedling mortality. The disease is considered one of the most destructive problems in groundnut cultivation, with yield losses ranging from 13 to 52 per cent and in severe cases reaching up to 93.6 per cent.

Barwant and Lavhate (2020) reported that *Aspergillus niger* and *Aspergillus flavus* commonly infect seeds of crops such as soybean, maize and gram. These fungi reduce germination, cause seed discoloration and shrivelling, and lead to yield losses. The pathogens were isolated from infected seeds and cultured on potato dextrose agar (PDA), and identification was confirmed through microscopic observation of spores.

Nguyen *et al.* (2023) studied collar rot of groundnut caused by *A. niger* in different regions of Vietnam. Diseased groundnut samples were collected from several locations and the associated fungal isolates were identified based on morphological and molecular characteristics. The isolates showed typical features of *A. niger*, such as black colonies, biseriate conidial heads and hyaline conidiophores. Pathogenicity tests confirmed that the isolates were capable of causing collar rot in *Arachis hypogaea*.

12.2 Synthesize and characterize nanoparticles for their physiochemical properties

12.2.1 Synthesis of silver nanoparticles (AgNPs)

Previous studies indicate that plant phytochemicals play an important role in the green synthesis of nanoparticles by acting as reducing, stabilizing and capping agents. Unlike conventional chemical synthesis methods that use toxic reagents, plant-based synthesis is considered safer and more environmentally friendly. Various plant parts such as leaves, stems, flowers, bark, roots and seeds, along with their metabolites, have been successfully used for nanoparticle production (Parashar *et al.*, 2009).

Logaranjan *et al.* (2016) demonstrated the controlled synthesis of silver nanoparticles using *Aloe vera* plant extract and evaluated their antimicrobial activity. Similarly, Karatoprak *et al.* (2017) produced silver nanoparticles from the extract of *Pelargonium endlicherianum*, where phytochemicals such as gallic acid, apocyanin and quercetin acted as natural reducing agents.

Saranya *et al.* (2018) also described an eco-friendly and economical method for synthesizing silver nanoparticles using leaf extract of *Ocimum*. The plant is known to contain phytochemicals such as alkaloids, glycosides, tannins and saponins, which act as natural reducers and stabilizers. The reduction of silver ions by the leaf extract resulted in the formation of well-defined silver nanoparticles.

Singh *et al.* (2018) reported the synthesis of silver nanoparticles using leaf extract of *Tulsi* under sunlight at room temperature. The study indicated that tulsi leaves are suitable for rapid and simple production of nanoparticles. Tulsi contains a complex mixture of nutrients and bioactive compounds among which eugenol and ursolic acid are considered major active constituents. These phytochemicals contribute to the reduction of silver ions and formation of nanoparticles.

Rolim *et al.* (2019) described the green synthesis of silver nanoparticles using extract of *Camellia sinensis* (green tea) through a simple one-pot method. The plant

extract functioned both as a reducing and stabilizing agent. The presence of tea polyphenols played an important role in converting silver ions into stable nanoparticles.

12.2.2 Synthesis of iron nanoparticles (FeNPs)

Atef *et al.* (2015) reported the biological synthesis of iron oxide nanoparticles using *Candida albicans*. The yeast culture was grown in broth at 37 °C for 24 hours. The biomass was collected and washed under sterile conditions, and about 20 g of wet biomass was suspended in 100 ml of aqueous solution containing ferric chloride ($FeCl_3$) and ferrous chloride ($FeCl_2$) in a molar ratio of 2:1, with pH adjusted to 3.1. The reaction mixture was incubated at 27 °C with shaking (200 rpm) for 24 hours. After the reaction, fungal biomass was removed by filtration, and the synthesized iron nanoparticles were recovered from the solution using magnetic separation.

Ghani *et al.* (2017) reported the biosynthesis of iron nanoparticles using *Bacillus megaterium*. The bacterium was grown in nutrient broth and incubated for 32 hours at 24 °C. The bacterial suspension was then mixed with 0.1 M $FeNO_3$ solution in a 1:1 ratio, resulting in rapid synthesis of iron nanoparticles at room temperature within about 20 minutes. The mixture containing nanoparticles was filtered through Whatman No.1 filter paper followed by a 0.22 µm microbiological filter to obtain purified nanoparticles.

Devatha *et al*. (2018) reported the green synthesis of iron nanoparticles using *Azadirachta indica* leaf extract and ferrous sulphate as a precursor. Different precursor to extract ratios ranging from 1:1 to 1:5 were evaluated for nanoparticle formation. The presence of polyphenols such as gallic acid (0.280 mg/g), caffeic acid (0.278 mg/g) and catechin (0.532 mg/g), along with a total phenolic content of about 20 mg/g in the leaf extract, played a key role in the reduction and stabilization of iron nanoparticles.

Zambri *et al.* (2019) reported the green synthesis of iron oxide nanoparticles (Fe_3O_4) using *Azadirachta indica* leaf extract as a natural reducing and stabilizing agent. XRD results indicated a highly pure cubic crystalline structure. TEM and FESEM analysis revealed nanoparticles with mostly irregular shapes and an average size of 9-12 nm. Energy dispersive X-ray analysis confirmed the presence of iron (33.29%) and oxygen (66.71%).

Rathore and Devra (2022) reported a green synthesis of iron nanoparticles using leaf extract of *Azadirachta indica*. The formation of FeNPs was monitored using UV-Visible spectroscopy. The optimum conditions for synthesis were 15% leaf extract, 1.0 mM $FeCl_3$ concentration, pH 6.0 and temperature of 60 °C.

12.2.3 Characterization of AgNPs by various instruments

Anandalakshmi *et al.* (2016) reported the synthesis of silver nanoparticles using *Pedalium murex* leaf extract. UV-Vis spectroscopy showed a characteristic absorption peak around 430 nm which slightly shifted to 424 nm during nanoparticle formation. FTIR analysis recorded peaks at 445, 617, 1075, 1287, 1421, 1602, 3157 and 3785 cm^{-1}, indicating the involvement of various functional groups. DLS analysis showed particle sizes ranging from 10 to 150 nm with an average size of 73.14 nm.

Rajoriya *et al.* (2016) reported the biosynthesis of silver nanoparticles using aqueous extracts of garlic (*Allium sativum*) and onion (*Allium cepa*). The formation of nanoparticles was confirmed by the appearance of a yellowish-brown colour. UV-Vis spectrophotometric analysis recorded absorption peaks between 350 and 550 nm at different time intervals under both light and dark conditions.

Dhanapal *et al.* (2017) reported the biosynthesis of silver nanoparticles using aqueous leaf extract of *Ocimum sanctum*. The addition of plant extract to silver nitrate solution caused the colour of the mixture to change to dark cherry red, indicating nanoparticle formation. UV-Vis analysis showed a characteristic absorption peak at 438 nm. FTIR spectra recorded in the range of 500–3500 cm^{-1} revealed peaks at 712, 1058, 1322, 1640, 2598 and 3270 cm^{-1}, which correspond to functional groups such as cyclic C–O–C, C=O and –OH, suggesting their role in nanoparticle synthesis and stabilization.

Alsammarraie *et al.* (2018) synthesized silver nanoparticles using turmeric extract. UV-Vis spectroscopy showed a maximum absorption peak at 432 nm. TEM analysis revealed mostly spherical nanoparticles with an average particle size of 18 ± 0.5 nm.

Hussain *et al.* (2018) synthesized silver nanoparticles using *Moringa oleifera* extract and recorded characteristic UV-Vis peaks between 413 and 420 nm. SEM images showed nanoparticles with rectangular morphology.

Jacob *et al.* (2018) studied AgNPs synthesized using *Ocimum sanctum* extract and observed a UV-Vis peak at 438 nm. SEM analysis indicated an average particle size of about 43 nm, while TEM revealed spherical nanoparticles with sizes ranging from 10 to 50 nm.

Premkumar *et al.* (2018) reported the synthesis of silver nanoparticles from cinnamon extract with UV-Vis absorption peaks between 410 and 425 nm.

Rolim *et al.* (2019) reported rapid one-pot synthesis of silver nanoparticles using *Camellia sinensis* extract. DLS analysis showed an average hydrodynamic size

of 34.68 ± 4.95 nm with a polydispersity index of 0.28 ± 0.01 and a zeta potential of −35.5 ± 3.32 mV, confirming the formation of stable and moderately dispersed nanoparticles.

12.2.4 Characterization of FeNPs by various instruments

Unsoy *et al.* (2012) prepared Fe_3O_4 nanoparticles using $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ with chitosan and tripolyphosphate as reducing and stabilizing agents. Particle size analysis showed an average size of 58 nm, while TEM images confirmed spherical nanoparticles with sizes between 18-20 nm. XRD results verified the formation of the Fe_3O_4 phase and FTIR analysis indicated the involvement of functional groups responsible for stabilization.

Mihir and Siddhivinayak (2015) synthesized iron nanoparticles using *Curcuma longa* extract with $FeCl_3 \cdot 6H_2O$ as the precursor. UV-Vis spectroscopy showed an absorption peak at 377 nm. XRD and FTIR analyses confirmed the crystalline nature and presence of functional groups responsible for nanoparticle stabilization. SEM analysis revealed cuboid and pyramidal shaped nanoparticles.

Pham *et al.* (2016) synthesized iron nanoparticles using $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ with chitosan as the stabilizing agent. XRD analysis showed a cubic crystalline structure with an average particle size of approximately 25 nm. SEM and TEM analyses revealed spherical nanoparticles with sizes ranging from 8-17 nm and 13-17 nm respectively.

El-Kassas *et al.* (2017) synthesized iron nanoparticles using *Padina pavonica* extract and $FeCl_3 \cdot 6H_2O$ as the precursor. XRD and FTIR analyses confirmed nanoparticle formation and stabilization by biomolecules. TEM analysis revealed spherical nanoparticles with sizes ranging from 10-27.4 nm.

12.3 Investigate the biochemical changes induced in groundnut upon nanoparticle treatment.

Attitalla and Alhendawi (2013) examined protein content in peanut grains and observed noticeable differences after contamination with *Aspergillus* species. Although protein levels between two varieties were not significantly different ($P < 0.05$), contamination duration significantly influenced protein content.

Gajera *et al.* (2014) also studied biochemical responses of groundnut varieties against collar rot caused by *Aspergillus niger*. Tolerant varieties (J-11 and GG-2) accumulated higher phenolic content than moderately susceptible (GAUG-10, GG-13)

and susceptible (GG-20) varieties. Phenol accumulation increased by 193% in GG-20, 146% in GG-2 and 107% in J-11 during disease development.

Rojasara *et al.* (2016) evaluated biomolecules such as soluble sugars, proteins and phenols in rust-resistant groundnut entries (PI-476177 and PI-476168). Susceptible entries (PI-476165 and J-11) accumulated these biomolecules at a higher rate during infection. Micronutrients such as copper and zinc were higher in resistant lines, whereas iron and manganese were greater in susceptible lines.

Parbat *et al.* (2018) investigated biochemical parameters in forty groundnut genotypes against tikka disease. Resistant genotypes exhibited higher phenol, sugar and chlorophyll contents compared to susceptible ones. During disease progression, phenol and sugar levels increased while chlorophyll content decreased.

Hebat-Allah *et al.* (2019) evaluated nano-selenium application (0, 20 and 40 ppm) on three groundnut cultivars (NC, Gregory and Giza-6) under sandy soil conditions. Nano-selenium improved yield components and seed oil content and also enhanced the proportion of unsaturated fatty acids while reducing saturated fatty acids compared with untreated plants.

12.4 Investigate role of epidemiological factors on disease development

Raju *et al.* (2003) reported that environmental factors play an important role in the development of collar rot of groundnut caused by *Aspergillus niger*. Disease incidence increased with rising temperature up to 35 °C, after which a decline was observed. Higher relative humidity also favored disease development, with maximum infection recorded at 100% relative humidity.

Kishore *et al.* (2007) observed that collar rot of groundnut is commonly associated with high temperature and low soil moisture conditions, with severe incidence occurring around 30 °C.

Bajaya *et al.* (2022) conducted a survey in eight districts of Rajasthan covering 200 groundnut fields and reported a mean disease incidence of 22.99%, ranging from 17.84% to 32.38%. The highest incidence was recorded in Jaipur district (28.85%), while the lowest was observed in Nagaur (20.15%). The study also indicated that increasing the seed rate to 105 kg/ha reduced disease incidence by 12.97% and increased pod yield by 27.65% compared to a lower seed rate of 80 kg/ha, possibly due to increased canopy density that moderated temperature and improved humidity within the crop canopy.

12.5 Evaluate the efficacy of different metal-based nanoparticles (AgNPs, FeNPs) against *Aspergillus niger* under *in vitro* condition.

12.5.1 Through AgNPs and FeNPs

Abdulrahaman and Hussein (2017) studied the antifungal activity of silver nanoparticles against *Aspergillus flavus*. The results showed that AgNPs effectively inhibited fungal growth on PDA medium, with 100% inhibition at concentrations of 175 and 200 ppm, and 95% inhibition at 150 ppm. HPLC analysis also confirmed that silver nanoparticles completely suppressed aflatoxin B_1 (AFB_1) production in maize grains contaminated with *A. flavus*, indicating their potential use in controlling fungal contamination in stored grains.

Alananbeh *et al.* (2017) investigated the antifungal effect of silver nanoparticles against fungi isolated from wastewater sources. A total of nine fungal species were identified, among which *Aspergillus* species were predominant. Silver nanoparticles of two shapes (rod and cube) were tested at concentrations of 0, 1, 10 and 100 µg/ml against *Aspergillus niger* and *A. terreus*. The results showed a gradual reduction in fungal growth with increasing nanoparticle concentration. *A. terreus* exhibited greater sensitivity compared to *A. niger*. Rod-shaped nanoparticles were more effective in inhibiting fungal growth than cube-shaped nanoparticles, indicating the potential use of silver nanoparticles as antifungal agents.

Parveen *et al.* (2018) synthesized iron oxide nanoparticles (Fe_2O_3) of 10-30 nm using tannic acid and evaluated their antifungal activity against several fungi including *Aspergillus niger*. The nanoparticles showed significant antifungal activity by inhibiting spore germination and fungal growth. The largest inhibition zone was recorded for *Penicillium chrysogenum* (28.67 mm) followed by *Aspergillus niger* (26.33 mm). The MIC values ranged from 0.016-0.063 mg/ml, indicating strong antifungal potential of Fe_2O_3 nanoparticles.

Koka *et al.* (2019) evaluated the antifungal activity of MgO and FeO nanoparticles against several fungi including *Aspergillus niger*. The nanoparticles significantly inhibited spore germination and mycelial growth at concentrations of 0.1, 0.25 and 0.5 mg/ml. However, the lowest inhibition was observed in *A. niger* compared to other tested fungi, indicating moderate sensitivity of this pathogen to MgO and FeO nanoparticles.

Abdelghany *et al.* (2020) studied the antifungal activity of *Juniperus procera* stem extract combined with silver nanoparticles against *Aspergillus flavus*. Methanolic

stem extract at concentrations of 30, 60 and 90 mg/ml inhibited fungal growth by 50.86%, 51.60% and 52.58%, respectively, whereas the aqueous extract showed lower inhibition. Silver nanoparticles alone showed a maximum growth inhibition of 39.31% at 100 ppm. A synergistic effect was observed when 50 ppm AgNPs were combined with plant extracts, which also reduced the production of aflatoxins B_2 and G_2. The study indicated that the combination of plant extract and silver nanoparticles can effectively suppress *A. flavus* growth and toxin production.

Molina-Hernandez *et al.* (2022) synthesized AgNPs using catechin, a natural polyphenol, and evaluated their antifungal activity against *Aspergillus niger*. AgNPs at 7.5 and 15 ppm induced physiological and metabolic stress in the fungus. The higher concentration (15 ppm) reduced mycelial biomass by 81%. The antifungal effect was associated with increased reactive oxygen species (ROS) production, oxidative stress and membrane damage, which inhibited fungal growth.

Shi *et al.* (2022) studied the effect of magnetic nanoparticles (Fe_3O_4) of different sizes (10, 20 and 200 nm) on the growth and sporulation of *Aspergillus niger*. The nanoparticles did not significantly affect fungal growth; however, MNP20 at 500 mg/L markedly reduced sporulation and spore production. The reduction in sporulation was associated with increased chitin accumulation in the fungal cell wall which indicating cell wall disruption caused by the nanoparticles.

Ansari *et al.* (2023) studied the effect of green-synthesized silver nanoparticles using neem leaf extract on tomato plants infected with *Alternaria solani*. AgNP treatment increased plant height by 30%, fresh weight by 45%, and dry weight by 40% compared to control plants. Disease severity index and disease incidence were reduced by 73% and 69% respectively. Application of 5-10 ppm AgNPs enhanced photosynthetic pigments and increased antioxidant enzyme activities such as PO (60%), PPO (65%), PAL (65.5%), SOD (65.3%), CAT (53.8%) and APX (73%) indicating improved plant growth and resistance against early blight disease.

Kamal *et al.* (2023) reported the green synthesis of iron and ZnO nanoparticles using *Daedalea* mushroom as a biological source. The nanoparticles were tested against *A. niger* and showed significant antifungal activity. Iron nanoparticles inhibited fungal growth by 60-72% at concentrations of 0.25-0.75 mg/mL, while ZnO nanoparticles showed maximum inhibition of 88% at 0.25 mg/mL. The study suggested that mushroom-mediated nanoparticles can serve as eco-friendly antifungal agents.

Periakaruppan *et al.* (2023) synthesized iron oxide nanoparticles using congress grass leaf extract through a green synthesis method. The nanoparticles were spherical with an average particle size of 17.5 nm and showed good stability and crystalline nature. The antifungal activity of these nanoparticles was tested against the fungal pathogens *A. niger* and *A. flavus*. The nanoparticles significantly inhibited the growth of both fungi under in vitro conditions. Antioxidant analysis showed a free radical scavenging activity with an IC_{50} value of 184.3 µg/ml. Seed germination tests conducted on sesame and groundnut indicated that lower concentrations of nanoparticles enhanced germination, suggesting their potential application as eco-friendly nanofungicides in crop protection.

Pirbalouti *et al.* (2024) reported that green-synthesized silver nanoparticles exhibited dose-dependent antifungal activity. The minimum inhibitory concentration (MIC) for *Candida albicans* was 80 µg/mL using the broth microdilution method. Plate assays showed significant growth inhibition of *A. niger* at 320 µg/mL and *Microsporum canis* at 80 µg/mL. These results confirmed the effectiveness of AgNPs in suppressing fungal growth at relatively low concentrations.

Uzair *et al.* (2025) synthesized cobalt–iron oxide ($CoFe_2O_4$) nanorods using a microwave-assisted solvothermal method and evaluated their antimicrobial activity. The nanorods showed significant antifungal activity against *A. niger* and *A. flavus* producing inhibition zones of 23 mm and 18 mm, respectively. The study indicated that cobalt–iron oxide nanoparticles possess strong antifungal potential and could be applied for microbial control.

12.6 Evaluate the efficacy of selected fungicides and nanoparticles in reducing disease incidence in groundnut under field conditions.

12.6.1 Through Nanoparticles

Kaur *et al.* (2018) evaluated the field/pot application of biosynthesized silver nanoparticles for the management of wilt disease of chickpea caused by *Fusarium oxysporum* f. sp. ciceri. The AgNPs (20-50 nm) were applied as seed treatment and showed high antifungal activity. Pot experiment results revealed that AgNPs reduced wilt incidence by 73.33% compared to 26.67% reduction with copper oxychloride. Seeds coated with AgNPs also showed higher germination up to 98% and no negative effect on soil microbial community was observed. The study indicated that biosynthesized silver nanoparticles are effective and eco-friendly for managing chickpea wilt disease and improving seed germination.

Sultana *et al.* (2021) investigated the effects of biogenic silver nanoparticles synthesized using *Moringa oleifera* leaf extract on rice plants infected with *Aspergillus flavus*. Characterization by UV-Vis spectroscopy, SEM and EDX confirmed crystalline AgNPs with sizes below 100 nm. Field application of AgNPs at concentrations of 25, 50, 75 and 100 mg/L showed that 50 mg/L was most effective in controlling fungal proliferation. The treatment improved physiological parameters such as relative water content, membrane stability index and chlorophyll content, and regulated biochemical and enzymatic metabolites. AgNP application reduced biotic stress and significantly increased protein content in rice plants.

Kumar *et al.* (2022) conducted a field study to evaluate the effect of silver nanoparticles on leaf curl disease of chili (*Capsicum annuum*) and leaf spot disease of okra (*Abelmoschus esculentus*). A foliar spray of 50 ppm AgNPs was applied at 10-12 days intervals for 10-12 weeks after transplanting. The treatment significantly reduced disease severity by 29.05% in chili and 23.2% in okra, whereas control plots showed higher disease severity of 67.15% and 54.9%, respectively. AgNP treatment also increased crop yield to 0.985 kg per plant in chili and 1.656 kg per plant in okra compared to 0.687 kg and 0.679 kg per plant in control plots. The biological efficiency of AgNP spray was recorded as 57% in chili and 55% in okra, indicating its effectiveness in managing plant diseases under field conditions.

Divya *et al.* (2023) evaluated the effect of green synthesized silver nanoparticles on leaf blight disease of red *amaranthus* (*Amaranthus tricolor*) caused by *Rhizoctonia solani*. The AgNPs (35-45 nm) were synthesized using leaf extracts of different *amaranthus* genotypes and applied as foliar spray. In vivo results showed that spraying Ad-AgNPs at 750 ppm recorded the lowest disease index of 7.40, followed by Av-AgNPs with a disease index of 17.69, while At-AgNPs treated plants showed a higher disease index of 49.38 five days after pathogen inoculation. The study indicated that AgNPs synthesized from resistant genotypes effectively reduced leaf blight disease in *amaranthus*.

Islam *et al.* (2024) conducted greenhouse and field experiments to evaluate the effect of green synthesized silver nanoparticles on sheath blight disease of rice caused by *Rhizoctonia solani*. The AgNPs were synthesized using *Ipomoea carnea* leaf extract with particle sizes ranging from about 45-73.81 nm and spherical shape. Field results showed that the application of AgNPs significantly reduced sheath blight disease and showed effectiveness comparable to commercial fungicides. The study suggested that

biosynthesized silver nanoparticles can serve as a sustainable and eco-friendly alternative for managing sheath blight disease in rice under field conditions.

Hegazy *et al.* (2025) conducted a greenhouse (field condition) study to evaluate the effect of silver nanoparticles on basal rot disease of onion. AgNPs (12.1-28.7 nm) were applied at concentrations of 50 and 100 ppm through foliar spray and soil application in a randomized complete block design. The results showed that 100 ppm AgNPs spray reduced disease severity to 22.2%, while soil application reduced it to 31.1%. The 50 ppm treatment reduced disease severity to 35.55% (spray) and 57.77% (soil). In addition to disease reduction, 100 ppm AgNPs significantly improved plant growth and increased chlorophyll a, chlorophyll b and carotenoid content. The study indicated that silver nanoparticles are effective in controlling basal rot disease and enhancing growth and physiological parameters of onion plants.

12.6.2 Through Fungicide

Nandeesha *et al.* (2013) assessed the efficacy of systemic fungicides such as carbendazim, propiconazole, tebuconazole and hexaconazole, along with non-systemic fungicides like mancozeb and captan against *A. niger*. The systemic fungicides were highly effective and completely inhibited the mycelial growth of the pathogen even at a concentration of 250 ppm.

Geetha *et al.* (2016) evaluated the effect of carbendazim, mancozeb and copper oxychloride against *Aspergillus niger*. Carbendazim showed the highest inhibition zone (37 mm), followed by copper oxychloride (22 mm) and mancozeb (12 mm).

Kapadiya and Moradiya (2017) evaluated chemical and biological control combinations for managing collar rot and stem rot diseases. The lowest collar rot incidence (2.64%) and stem rot incidence (1.27%) were recorded with seed treatment of tebuconazole (1.5 g/kg) followed by two sprays of the same fungicide at 15-day intervals. This treatment also resulted in higher pod and haulm yields.

Rani *et al.* (2017) tested several fungicides and herbicides against *Aspergillus niger* and observed maximum radial growth inhibition (100%) with tebuconazole. Mancozeb + carbendazim also showed high inhibition (92.94%) of fungal growth.

Sekhon *et al.* (2019) evaluated six fungicides using poisoned food technique and paper towel method against *A. niger*. Tebuconazole at concentrations of 25, 50, 100 and 200 ppm and azoxystrobin at 100 and 200 ppm completely inhibited fungal growth. Carbendazim + mancozeb (200 ppm) also showed high inhibition (94.38%). Seed

treatment with tebuconazole resulted in the lowest seed mortality and seed rot in groundnut varieties SG-99 and M-522.

Piprotar *et al.* (2022) evaluated different groups of fungicides against *A. niger* under *invitro* and field conditions. Among non-systemic fungicides, copper oxychloride showed complete inhibition at 1000-2000 ppm, while among systemic fungicides carbendazim, propiconazole and tebuconazole achieved 100% inhibition at 100–500 ppm. In field trials, seed treatment with carboxin 37.5% + thiram 37.5% (3 g/kg seed) recorded the lowest disease incidence (3.96%) and higher pod yield (1766 kg/ha), followed by fluxapyroxad + pyraclostrobin.

13. Materials and Methods

The proposed research work entitled "Nanoparticle-Mediated Induction of Defense Responses in Groundnut (*Arachis hypogaea*) against *Aspergillus niger* van Tieghem causing Collar Rot" will be carried out at laboratory and experimental fields of Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh. The details of various materials used and the methodology followed are described as under.

13.1 Isolate, purify, identify and confirm the pathogenicity of fungus causing collar rot of groundnut.

13.1.1 Collection of diseased samples

During the *Kharif* season of 2026-27, groundnut plants exhibiting typical symptoms of collar rot will be collected from the field and brought to the laboratory for detailed investigation.

13.1.2 Isolation and purification

Fresh, naturally infected groundnut plants showing characteristic collar rot symptoms will be selected for pathogen isolation on PDA medium. The diseased samples will be washed thoroughly under running tap water to remove adhering soil and debris, followed by blotting to remove excess moisture. Small pieces (3-4 mm) of infected root tissue along with a portion of healthy tissue will be cut using a sterilized blade.

The tissue pieces will be surface sterilized in 1% sodium hypochlorite solution for one minute and subsequently rinsed three times with sterile distilled water to eliminate traces of disinfectant. The sterilized pieces will be dried on sterile blotter paper and aseptically transferred onto Petri plates containing PDA medium. The plates will be incubated at 25 ± 1°C in a BOD incubator.

After sufficient fungal growth (approximately 72 hours), purification will be carried out using the hyphal tip culture method as described by Riker and Riker (1936). A small agar disc containing fungal growth will be transferred to fresh agar medium and incubated for two days. Under a dissecting microscope, actively growing hyphal tips will be identified, excised (about 1 mm from the apex), and transferred to fresh agar plates to obtain pure cultures. The purified isolates will be maintained and preserved for further experimental studies

13.1.3 Identification

The fungal isolate will be identified based on its cultural and morphological characteristics. The pathogen is expected to produce septate, hyaline to pale yellow hyphae. Colonies will typically appear black in color with a dull white or colorless reverse side. Conidiophores vary in length, diameter and may be hyaline, smooth, septate or non-septate.

The fungus will show rapid vegetative growth on culture media with profuse submerged mycelium. Conidial heads will range from small, columnar forms to large, globose or radiate structures. Conidia with rough-walled, dark brown to black and globose to sub-globose in shape and vesicles will be globose (Gilman, 2001).

13.1.4 Multiplication of inoculum

For mass multiplication, the test fungus will be cultured on sorghum grains following the method described by Gupta and Kolte (1982). Sorghum grains will be soaked overnight in water and excess water will be drained off. Approximately 200 g of soaked grains will be placed in 500 ml conical flasks, plugged with cotton, and sterilized in an autoclave at 1.045 kg/cm² pressure for 20 minutes.

After cooling to room temperature, the sterilized grains will be inoculated aseptically with 3-4 day-old actively growing mycelial discs (5 mm diameter) of *A. niger*. The inoculated flasks will be incubated at 25 ± 1°C for 10 days to allow uniform fungal growth and colonization of the substrate.

13.1.5 Pathogenicity test

The pathogenicity of the isolated and purified fungus will be tested using the seed inoculation technique as described by Kataria and Grover (1976). Apparently healthy, surface-sterilized groundnut seeds of a susceptible variety will be used for the experiment. The seeds will be rolled over a seven-day-old sporulating culture of *A. niger* grown on PDA plates to ensure uniform inoculation.

Four inoculated seeds will be sown at a depth of 5 cm in each pre-sterilized earthen pot containing autoclaved soil with three replications maintained for each treatment. Uninoculated healthy seeds will serve as the control. The pots will be maintained in a cage house and watered as required. Observations on disease development and seedling mortality will be recorded up to 20 days after sowing to confirm the pathogenic nature of the isolate.

13.2 Synthesize and characterize nanoparticles for their physiochemical properties

13.2.1 Preparation of neem leaf extract

The leaf of neem will be collected from Junagadh campus. The leaves will be washed thoroughly with distilled water and subsequently dried to remove moisture completely. After drying, leaves will be boiled with double quantity of double distilled water at 80°C for 60 minutes. The resulting product will be filtered first through a muslin cloth and then using Whatman No. 1 filter paper and centrifuge at 10,000 rpm for 10 minutes and finally, leaf extract will be store in the refrigerator for further study (Gopalakrishnan and Muniraj 2021).

13.2.2 Synthesis of silver nanoparticles (AgNPs)

A 1 mM silver nitrate ($AgNO_3$) solution will be prepared by dissolving 0.17g of silver nitrate in 1 L of distilled water in a beaker. Neem leaf extract known for its reducing properties will be mixed with the $AgNO_3$ solution in a ratio of 1:9 and transferred into a conical flask. The flask containing the reaction mixture will be heated in a water bath maintained at 80°C. During the reaction a distinct color change from yellow to brown will be observed that indicating the formation of silver nanoparticles. To remove any insoluble impurities the reaction mixture will be subjected to centrifugation at 3000 rpm for 10 minutes. The obtained precipitate will be washed thoroughly with ethanol, acetone and distilled water to ensure purification. Finally, the purified samples will be transferred to Petri dishes and dried at 90°C for 4 hours in a water bath (Jervin Ananth *et al*., 2023).

13.2.3 Synthesis of iron nanoparticles (FeNPs)

A 1mM iron salt solution will be prepared by dissolving the required amount of ferric chloride ($FeCl_3$) or ferrous sulfate ($FeSO_4$) in de-ionized water under continuous stirring. Neem leaf extract will be added dropwise to the iron salt solution in a 1:5 ratio under constant stirring at room temperature. The reaction mixture will be heated at 70-80°C for 30-60 minutes. A visible color change from yellowish/brown to dark brown

or black will indicate the formation of iron nanoparticles due to the reduction of Fe^{3+}/Fe^{2+} ions by phytochemicals present in the neem extract. The reaction mixture will be centrifuged at 10,000-12,000 rpm for 15-20 minutes. The obtained precipitate will be washed repeatedly with distilled water and ethanol to remove impurities. The purified nanoparticles will be dried at 60-80°C in a hot air oven for further characterization (Rathore and Devra, 2022).

13.2.4 Characterization of green synthesized Nanoparticles

Following the successful biosynthesis process, the synthesized silver nanoparticles (AgNPs) and iron nanoparticles (FeNPs) will be systematically characterized using advanced analytical techniques like UV-Visible spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM).

Observations to be Recorded

- UV-Visible Spectroscopy: Observation of absorbance and confirmation of AgNPs and FeNPs formation through characteristic surface plasmon resonance (SPR).
- XRD: Determination of crystallinity of AgNPs and FeNPs
- SEM: Shape, surface morphology, particle size range and degree of aggregation.

13.3 Investigate the biochemical changes induced in groundnut upon nanoparticle treatment.

Experiment details

Location	**:**	PG Laboratory, Department of Plant Pathology, COA, JAU, Junagadh
Season and Year	**:**	*Kharif*, 2026 and 2027
Crop	**:**	Groundnut
Variety	**:**	GG 20

Biochemical studies will be conducted to evaluate the effect of silver nanoparticles (AgNPs) and iron nanoparticles (FeNPs) on defense-related metabolites in plant roots. Groundnut root samples will be collected at 45 days after sowing (DAS) from treated healthy plants and untreated diseased plants. The biochemical parameters like total phenols, soluble proteins and total sugars will be analyzed using standard protocols.

13.3.1 Estimation of Total Phenols

Total phenol content will be determined following the method of Thimmaiah (1999).

13.3.2 Estimation of Soluble Proteins

Soluble protein content will be estimated by the Lowry method (Lowry *et al*., 1951).

13.3.3 Estimation of Total Sugars

Total sugars will be estimated by the phenol-sulphuric acid method (Du Bois *et al*., 1956).

13.4 Investigate role of epidemiological factors on disease development

Experiment details

Location	**:**	Research Farm, Department of Plant Pathology, COA, JAU, Junagadh
Season and Year	**:**	*Kharif*, 2026 and 2027
Crop	**:**	Groundnut
Variety	**:**	GG 20
Plot Size	**:**	20 × 20 m^2
Spacing	**:**	60 cm × 10 cm

Epidemiology involves the study of factors responsible for the occurrence, development and spread of plant diseases. The incidence and severity of a disease depend on the interaction among a susceptible host, a virulent pathogen, and favorable environmental conditions. In groundnut, collar rot caused by *A. niger* is an important disease during the *Kharif* season and is greatly influenced by weather parameters such as temperature, relative humidity, rainfall, rainy days and sunshine hours.

Weekly observations on plant mortality and disease incidence will be recorded from germination to maturity. Simultaneously, meteorological data such as temperature, relative humidity, rainfall, rainy days and sunshine hours will be collected from Agrometeorological Cell, Department of Agronomy, JAU, Junagadh.

Statistical analysis:

Further, multiple regression analysis will be carried out with weather parameters in relation to PDI. The relationship of disease with weather parameters will be established by following multiple linear regression equation.

$$Y = a+b_1X_1+ b_2X_2+ b_3X_3+ b_4X_4+ b_5X_5+ b_6X_6$$

Where,

Y = Disease incidence,

a = Intercept (constant),

b_1 to b_6 = Partial regression coefficient associated with each x_i,

i = 1, 2, 3, 4, 5, 6 are the weather variables,

X_1 = Maximum temperature (˚C),

X_2 = Minimum temperature (˚C),

X_3 = Morning relative humidity (%),

X_4 = Afternoon relative humidity (%),

X_5 = Rainy days (mm),

X_6 = Rainfall (mm)

The linear relationship of disease development with environmental factors was calculated by Karl Pearson linear correlation coefficient. The quantity r, called the linear correlation coefficient, measures the degree and direction of a linear relationship between two variables.

The mathematical formula for computing r is:

$$r_{xy} = \frac{\sum xy}{\sqrt{\sum x^2 \sum y^2}}$$

Where,

$\sum xy = n\sum XY - (\sum X)(\sum Y)$

$\sum x^2 = n\sum X^2 - (\sum X)^2$, $\sum y^2 = n\sum Y^2 - (\sum Y)^2$

n = number of pairs of data

r = Correlation coefficient

x = Independent variable (Abiotic factors)

y = Dependent variable (Per cent disease incidence)

$$t = \frac{r\sqrt{(n-2)}}{\sqrt{1-r^2}}$$

Where,

t = Test of significance

r = Correlation coefficient and

n = Number of observations

13.5 Evaluate the efficacy of different nanoparticles (AgNPs, FeNPs) against *Aspergillus niger* under *in vitro* condition

13.5.1 Details on *in vitro* evaluation of nanoparticles

Location	**:**	Department of Plant Pathology, COA, JAU, Junagadh
Year	**:**	*Kharif,* 2026-27

Experimental design	:	FCRD (Factorial Completely Randomized Design)
Treatments	:	First factor: 2 nanoparticles. Second factor: Concentration with four levels. Thus, Total treatments combination = 8 +1 (control) = 9 treatment combination. Control plate will be maintained to compare the radial growth of test pathogen with respect to treated plates to work out percent inhibition of mycelial growth.
Repetition	:	3

Table 1. Details of nanoparticle treatments

Tr. No.	Treatments	Concentration (ppm)			
T_1	Ag NPs	25	50	100	150
T_2	Fe NPs	25	50	100	150

13.5.2 Details on *in vitro* evaluation of fungicide

Location	:	PG Laboratory, Department of Plant Pathology, COA, JAU, Junagadh
Year	:	*Kharif,* 2026-27
Experimental design	:	FCRD (Factorial Completely Randomized Design)
Treatments	:	First factor: 5 fungicides. Second factor: Concentration with four levels. Thus, Total treatments combination = 20+1 (control) = 21 treatment combination. Control plate will be maintained to compare the radial growth of test pathogen with respect to treated plates to work out percent inhibition of mycelial growth.
Repetition	:	2

Table 2. Details of fungicide treatments

Tr. No.	Treatments	Concentration (ppm)			
T1	Hexaconazole 5 SC	100	200	300	400
T2	Carbendazim 50 WP	100	200	300	400

T3	Mancozeb 40 + Tebuconazole 1 WS	100	200	300	400
T4	Prochloraz 5.7 + Tebuconazole 1.4 w/w ES	100	200	300	400
T5	Tebuconazole 50 + Trifloxystrobin 25 WG	100	200	300	400

Methodology

The *in vitro* antifungal efficacy of silver nanoparticles (AgNPs) and iron nanoparticles (FeNPs) will be assessed against *Aspergillus niger* using the poisoned food technique on Potato Dextrose Agar (PDA) medium.

Required concentrations of AgNPs and FeNPs (25, 50, 100 and 150) will be incorporated separately into sterilized and molten PDA medium under aseptic conditions. For comparison, a standard fungicide treatment at 100, 200, 300 and 400 ppm will also be prepared by mixing the calculated quantity into sterilized PDA. The amended media will be poured into sterile Petri plates and allowed to solidify at room temperature.

A seven-day-old pure culture of *A. niger* will be used as the test pathogen. Using a sterile cork borer, a 5 mm diameter mycelial disc will be cut from the actively growing margin of the culture under laminar airflow conditions. The disc will be aseptically transferred to the centre of each treated Petri plate with the help of a flame-sterilized inoculation needle. Plates containing PDA medium without nanoparticles or fungicide, but inoculated with the pathogen will serve as the untreated control.

Each treatment will be replicated three times and incubated at 27 ± 2°C. Radial mycelial growth will be recorded at regular intervals until the fungal colony in the control plates completely covers the Petri plate. The percentage inhibition of mycelial growth over control will be calculated to determine the comparative antifungal effectiveness of AgNPs and FeNPs against *A. niger.*

Observations to be recorded

The per cent growth inhibition of the fungus in each treatment will be calculated by using following formula (Vincent, 1947):

$$PGI = \frac{C - T}{C} \times 100$$

Where,

PGI = Per cent growth inhibition

C = Average diameter (mm) of mycelial colony in control plate

T = Average diameter (mm) of mycelial colony in treated plate

13.6 Evaluate the efficacy of selected fungicides and nanoparticles in reducing disease incidence in groundnut under field conditions.

Table 3. Experimental Details for field evaluation

Location	**:**	Research Farm, Department of Plant Pathology, JAU, Junagadh
Season and Year	**:**	*Kharif*, 2026-27 and 2027-28
Crop	**:**	Groundnut
Variety	**:**	GG 20
Experimental Design	**:**	RBD (Randomized Block Design)
Treatment (No.)	**:**	Decided based on results of *in vitro* trials
Replication (No.)	**:**	Decided based on numbers of treatments included in trials
Plot Size	**:**	5.0 × 3.60 m^2
Spacing	**:**	60 cm × 10 cm

Methodology

The field experiment will be conducted in a Randomized Block Design (RBD) at the Research Farm, Department of Plant Pathology, JAU, Junagadh during *Kharif* season of 2026-27 and 2027-28 with eight treatments and three replications using susceptible groundnut variety GG-20. Healthy and uniform seeds of groundnut cv. GG-20 will be treated with respective treatments prior to sowing. The treated seeds will be sown directly in well-prepared field plots following recommended agronomic practices. Foliar application of treatments will be carried out twice during the crop growth period. The first spray will be applied at the initiation of disease symptoms, and the second spray will be given 15 days after the first spray. The concentration of treatment will be select based on *in vitro* studies, wherein the most effective concentration showing maximum inhibition of fungal growth will be used for the field experiment.

Observations on disease incidence and yield parameters will be recorded periodically to assess the effectiveness of treatments.

Observations to be recorded

Percent disease incidence (PDI), disease control and percent yield increase in experiments will be calculated as follows (Wheeler, 1969; Pradhan, 1969)

$$\text{Disease incidence (\%)} = \frac{\text{Number of Diseased plants}}{\text{Total number of plants observed}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{PDI in inoculated control-PDI in treatment}}{\text{PDI in inoculated control}} \times 100$$

$$\text{Yield increase (\%)} = \frac{\text{Yield in treated plot-Yield in control plot}}{\text{Yield in treated plot}} \times 100$$

14. Statistical analysis

Statistical analysis will be carried out as per standard method (Panse and Sukhatme, 1985).

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